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Vegetable Dust and Airway Disease: Inflammatory Mechanisms

by J. Allen D. Cooper, Jr.,* Marion G. Buck,* and J. Bernard L. Gee*†

Exposure to cotton or grain dust causes an obstructive bronchitis in certain subjects, mechanisms of which are poorly understood. A difficulty encountered in discerning mechanisms of this airway disease is the lack of knowledge of the active components of these dusts. Clinical features suggest common but not exact mechanisms of the airway disease associated with these vegetable dusts. Human and animal studies show evidence of acellular and cellular inflammatory mechanisms of the bronchoconstriction and inflammation associated with these disorders. Potential cellular sources include alveolar macrophages, polymorphonuclear leukocytes, mast cells, basophils, eosinophils and lymphocytes. Acellular origins include the complement and humoral antibody systems, both of which have been implicated, although their pathogenic role in grain or cotton dust disorders is uncertain. In this review we critically address potential inflammatory mechanisms of airway alterations resulting from cotton or grain dust exposure. General mechanisms of bronchoconstriction are first presented, then specific studies dealing with either of the two dusts are discussed. We believe this area of research may be fruitful in dissecting mechanisms of bronchoconstriction and airway inflammation, especially as more human studies are undertaken.

The primary focus of this conference is on cotton dust and related disorder(s). It is, however, appropriate to consider cotton dust together with another common economically important vegetable dust associated with airway disease, namely that associated with the harvesting, storage, and processing of grain. This review will address potential inflammatory mechanisms for some of the respiratory diseases associated with exposure to these dusts. We will first discuss similarities and differences of the patterns of the pulmonary response to these dusts. Secondly, we will review potential cellular and acellular inflammatory mechanisms in these disorders. General mechanisms of airway alterations will be reviewed, and evidence for a role in cotton or grain associated diseases will be discussed.

Before making specific comparisons between pulmonary responses to these two dusts, we should point out that neither grain nor cotton dusts are simple homogeneous systems. Aside from botanical variations in individual cotton or grain plants, there is a wide range of associated compounds acquired during the harvesting or processing procedures. These include bacteria, fungi, processing chemicals, and perhaps insects. The lack of knowledge of the active component(s) of either of these dusts makes human and animal experimental work regarding mechanisms very difficult. Thus, experimental studies have been performed with crude cotton or grain dust, bacteria, or endotoxin produced by bacteria isolated from these dusts, and proposed active chemical components of cotton, e.g., tannins or oils.

*Department of Internal Medicine, Pulmonary Section, Yale University School of Medicine, P. O. Box 3333, 333 Cedar St., New Haven, CT 06510.

†Author to whom all reprint requests and correspondence should be addressed.

Comparative Clinical Features

There is ample evidence to indicate that both cotton and grain dust exposure are associated with a high prevalence of cough and sputum. As such, the evidence for chronic nonobstructive bronchitis is clear, consistent and generally accepted. Likewise, both dusts are associated with chest tightness and both show enhancement of the foregoing symptoms by cigarette smoking. Finally, exposure to both dusts can result in febrile episodes whose nature is not completely understood (*1,2*). Discriminating features are that grain, in contradistinction to cotton, can invoke skin rashes and mucus membrane involvement, notably conjunctivitis, rhinitis and pharyngitis (*1*), both of which are very uncommon in cotton workers. There is a vague suggestion from one study (*3*) that there is a higher prevalence of heterozygote serum α-1-antiproteases with a slightly increased prevalence of MZ α-1-antiprotease phenotype in grain workers developing respiratory symptoms. However, this has not been fully confirmed and the significance is unclear; no such studies have been performed in cotton workers.

There are also some similarities and differences in the

pulmonary physiologic response to inhalation of cotton or grain dust (*1,2,4*). Asthma in the florid and easily recognized form is common among grain workers. Simple sifting of grain in front of a sensitized worker is enough to produce sharp falls in FEV_1, readily reversible with isoproterenol (*1*). In fact, most of the reactions to grain dust are of relatively acute onset, being manifest within minutes to an hour of exposure. More delayed responses up to several hours have also been reported in grain workers (*4*), but these are decidedly rare. This is in contrast to the classic features described for byssinosis in cotton workers (*2*). In general, the response time of a reduction in flow rates is measured more in hours and may require prolonged exposure time. The classic account is of the subject who goes to work well on Monday, after a weekend away, and throughout the day progressively deteriorates with a significant fall in FEV_1 over the shift. There is also a tendency for this phenomenon to worsen at the beginning of the work and progressively improve throughout the week suggesting induction of a tolerant state.

An additional distinction between cotton and grain effects on airway function is suggested by the use of histamine provocation tests. Some studies (*2,5*) in cotton workers with byssinosis do not indicate an increased sensitivity to histamine, while other studies (*1,6*) have shown an increased histamine airway responsiveness in symptomatic grain workers. One study (*7*) of airway reactivity in normal subjects reported a correlation between airway response to cotton bract extracts and methacholine but not with histamine responses. This finding is somewhat anomalous as both drugs yield similar responses in asthmatics and since histamine has been invoked in the etiology of byssinosis. Finally, there is considerable debate even now, after prolonged study, as to whether there is chronic loss of FEV_1 over many years as subjects continue to work in these dusty environments. This is not the place to review this in detail. Studies of grain workers have been extensively reviewed in a recent book (*8*), and two opposing accounts of the prevalence of excessive chronic FEV_1 loss in cotton workers have appeared in a recent book on occupational lung disease (*9,10*).

These findings suggest that there are certain similarities and differences in the airway response to grain and cotton. The next section of this review will discuss evidence for mechanisms of these effects and will be divided into specific cellular or noncellular inflammatory mechanisms of cotton- or grain dust-associated pulmonary disease. However, these mechanisms are not mutually exclusive and as noted, multiple mechanisms may be important. Work along these lines is very active, yet immature in many respects. This is particularly true in the instance of grain dust associated disease, as there are very few studies dealing with basic mechanisms of the airway alterations. In addition, although there is considerable literature dealing with cotton dust associated airway disease, the apparent multiplicity of mechanisms has made this a very complex and intriguing field. We will first provide overview sections of basic mechanisms of pulmonary manifestations occurring with exposure to cotton or grain dust, then review information of specific cellular or noncellular inflammatory mechanisms of these disorders. Except where noted, experimental work has been performed in animal models.

Mechanisms of Bronchoconstriction

In addition to airway smooth muscle contraction, other mechanisms frequently contribute to airway obstruction. These additional features certainly occur in classical extrinsic asthma and may also be involved in some of the reactions to cotton and grain dusts. They include changes in airway mucosa permeability, increased mucus production, and an accumulation of inflammatory cells in the airway. All of these airway disorders must be considered as inflammatory responses that are normal defense reactions of the lung to variably hazardous inhaled agents. As will become apparent, however, they are nonspecific. These inflammatory responses are observed not only in diseases associated with airway narrowing, but also occur with infectious and fibrogenic disorders. While the effects of such inflammatory agents clearly depend on their site of occurrence, this nonspecificity must be considered before any given inflammatory response can necessarily be inculpated in the pathogenesis of cotton or grain dust diseases.

Mediators of Airway Smooth Muscle Contraction

Acute airway narrowing due to smooth muscle contraction may occur as a result of the liberation of a number of mediators by pulmonary cells. Histamine causes airway narrowing by stimulating smooth muscle contraction or through indirect effects on cholinergic airway innervation (*11*). In addition, there is evidence (*12*) that histamine stimulates secondary release of arachidonic acid metabolites, specifically prostaglandin F_2 ($PGF_{2\alpha}$) from contracting airway smooth muscle. This bronchoconstricting prostaglandin may further enhance airway narrowing.

Other arachidonic acid metabolites produced by a variety of inflammatory cells are important bronchoconstricting agents. Prostaglandins, including $PGF_{2\alpha}$, PGD_2, and thromboxane A_2, may cause bronchoconstriction through effects on smooth muscle (*11*). In addition, lipoxygenase products of arachidonic metabolism, leukotrienes, and monohydeoxyeicosatetraenoic acids (monoHETEs) are bronchoconstrictors being on a molar basis, up to 2000 times more potent *in vitro* than histamine (*13*). Finally, another arachidonic acid product, acetylglyceryletherphosphorylcholine (platelet activating factor), produces changes in airway caliber indirectly through stimulation of the production of kinins by platelets (*14*) and release of arachidonic acid metabolites by other inflammatory cells (*15*). These platelet-produced kinins may have multiple effects, although contraction of

smooth muscle is probably their primary bronchoconstricting effect (*11*).

Mediators of Increased Mucosal Permeability

The innervation of bronchial smooth muscle is not readily accessible to mediators released by airway cells as an epithelial barrier separates them (*16*). In addition, the majority of mast cells, important in liberation of bronchoconstricting mediators, lie submucosally, not readily accessible to inhaled substances (*11*). Increased airway mucosal permeability may provide better access for mediators to smooth muscle receptors and for precipitating stimuli to mast cells (*17*). In addition, edema of the airways associated with this increased permeability may contribute to airway narrowing. Histamine, arachidonic acid metabolites including leukotrienes and prostaglandin E, kinins, and oxidants such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical may be important in mediating these effects. However, to date such alterations in mucosal permeability have not been demonstrated in bronchoconstrictive conditions such as asthma (*16*).

Mediators of Increased Mucus Production

Increased mucus production, commonly present in obstructive lung disease, may contribute to airway narrowing (*11*). Patients reacting to cotton or grain dust inhalation commonly show increased secretions suggesting increased mucus production. Mediators liberated by inflammatory cells which may induce increased mucus gland activity (*18*) include histamine; prostaglandins (PGA_2, PGD_2, PGE_1 and $PGF_2\alpha$; leukotrienes D_4 and C_4); cholinergic and α-adrenergic neural hormones; and also a newly described low molecular weight substance secreted by macrophages (*19*). All of these substances appear to increase mucus secretion by direct actions on mucus glands.

Specific Cellular Mechanisms

Alveolar Macrophage (AM)

This cell releases a number of bronchoconstrictive and inflammatory products. Some of these (prostaglandins, platelet-activating factors, chemotactic factors for polymorphonuclear leukocytes, oxidants, and interleukin-I) have been implicated in cotton or grain dust-induced pulmonary disease. However, while AMs release leukotrienes, their maximal production appears to be less than that of mast cells. Further, AMs release predominantly LTB_4 rather than the more powerful bronchoconstricting leukotrienes, LTC_4, LTD_4, or LTE_4 (*20*). As noted previously, these cells also secrete a low molecular weight mucus secretogogue (*19*).

AMs may cause airway inflammation and increased permeability by releasing directly toxic products and indirectly by releasing polymorphonuclear leukocyte chemotaxins. The former include proteolytic enzymes, such as neutrophil elastase and reactive oxygen metabolites (*21*), including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^*). All of these substances may cause direct epithelial injury, potentially resulting in bronchospasm through increased availability of submucosal mast cells to activating substances and increased exposure of smooth muscle receptors to mediators as already discussed.

In vitro, AMs also modulate the function of other inflammatory cells. First, they secrete such chemotactic factors for polymorphonuclear leukocytes (PMN) as a protein with a molecular weight of 10,000 daltons (*22*) and a low molecular weight lipid, probably LTB_4 or mono-HETEs (*21*). In addition, AMs may activate complement-producing components that are also chemotactic for PMN (*23*). Secretion of any of these factors *in vivo* should cause an influx of PMNs into the airways. Second, in response to a number of triggering agents, including endotoxin, AMs have been shown to release interleukin-1 (IL-1), a low molecular weight protein which enhances pulmonary inflammatory reactions through a number of pathways including propagation of T lymphocytes (*24*). Finally, a recent study (*25*) has suggested that pulmonary macrophages secrete a factor (>2000 MW) which stimulates the release of histamine from mast cells and basophils.

Information linking the AM specifically to cotton- or grain-associated pulmonary diseases is circumstantial. Several studies (*26–28*) have shown *in vivo* airway influx of PMNs in animals after one exposure to cotton dust extracts by inhalation. Although such infiltration may be due to release of macrophage-derived chemotactic factors, other inflammatory cells produce similar factors (*11,21*) and cotton dust itself contains PMN chemotaxins (*29*). We have shown (*30*) release of chemotactic factors for PMN from cultures of human AMs, obtained after normal subjects have inhaled an aqueous cotton bract extract. In addition, other investigators (*31*) have shown that tannins associated with cotton can stimulate *in vitro* release of PMN chemotaxins by AM. Thus, the alveolar macrophage is probably partially responsible for the influx of PMNs into the airways seen in these models of byssinosis. However, as will be described, the role of PMNs in the bronchoconstricting process is unclear. Such nonbronchoconstricting materials as asbestos also evoke AM release of PMN chemotaxins (*32*).

The relation between macrophage-derived bronchoconstrictors and cotton or grain dust has been examined only in animal studies. Rat peritoneal macrophages increase cyclic GMP concentrations and release prostaglandin $F_2\alpha$ after exposure to water-soluble extracts of several bacteria or fungi obtained from cotton (*33*). In addition, another laboratory (*34*) reports stimulation of prostaglandin $F_2\alpha$ release from rabbit AMs exposed to cotton dust extracts *in vitro*. Further studies suggested these effects were not due to endotoxin; endotoxin-resistant C34/HeJ mice responded similarly. Finally, a recent abstract (*35*) states that gossonorol, a cotton oil, stimulates rabbit AMs to release prostaglandin $F_2\alpha$ *in vitro*. However, after inhalation of the gossonorol, bron-

choalveolar lavage prostaglandin concentrations were not elevated. This last study emphasizes the difficulties in extrapolating *in vitro* findings to *in vivo* effects. Beijer and Rylander report that components of cotton stimulate the production of platelet-activating factor by bronchoalveolar lavage cells (*36*) from guinea pigs. Although certain components of cotton appear to show intrinsic platelet-activating factorlike properties, this study measured intracellular concentrations, suggesting cellular production of this agent.

There have been two animal studies (*37,38*) examining the effect of cotton constituents on macrophage oxidant production *in vitro*; one study also examined the effects on macrophage phagocytic prowess. In the most recent study (*37*), gossypol, rutin, and catechin, three chemical components of the cotton plant, were shown to inhibit rat AM functions including oxygen uptake, O_2^- production, and phagocytosis. Two problems exist with this study. First, all measurements were done in the presence of these cotton associated chemicals which may have oxidant scavenging properties. We have found that cotton bract extracts, which may contain similar chemicals, are oxidant scavengers (unpublished observations) and therefore may adversely affect *in vitro* measurement of cellular oxidant production. Second, estimation of cellular viability was provided by measuring cell volume alone, an insensitive test of cell injury.

The other study (*38*) reported an inhibition of AM luminol-dependent chemiluminescence by cotton bract extracts. Although this test purports to measure cellular oxidant production, it is a nonspecific assay, particularly in mitochondria-rich AMs. The authors of both of these studies proposed that inhibition of these macrophage bactericidal processes may increase bacterial growth and subsequently increase endotoxin production which has been implicated in byssinosis. In contrast, we have noted (*30*) an enhancement of the *in vitro* O_2^- production by human AMs obtained following the inhalation of a cotton bract extract. In our study, cells were washed several times prior to the O_2^- assay to minimize scavenging by components of the inhaled aqueous cotton extract.

AM effects on other inflammatory cells in byssinosis or grain-associated disease have also been examined. One study (*39*) claimed stimulation of rat AM interleukin-1 (IL-1) production by grain and cotton extracts, grain extracts being more potent than comparable cotton extracts. Since endotoxin is a powerful stimulator of IL-1 production in other systems, it is disturbing that IL-1 production did not correlate with the endotoxin levels present in these samples.

At autopsy, many patients "reported to have worked in cotton mills" show mucus gland hyperplasia and hypertrophy (*40*). Many mediators may cause such stimulation; those derived from macrophages include the low molecular weight protein already described, leukotrienes, HETE, and prostaglandins. However, to date no direct evidence has shown a link between production of these macrophage-derived mediators and mucus gland abnormalities in byssinosis.

As can be seen, evidence linking the AM to the primary inflammatory mediator in cotton- or grain-associated airway alterations is incomplete. The difficulty encountered in making this association is that there are currently no mediators that are specific for macrophage involvement. The possibility of contamination with other cells in cell cultures is a definite problem and difficult to overcome. Rylander (*41*) has proposed that in cotton-associated pulmonary disease, macrophages are the initiating cells in a process that culminates in PMN influx and platelet activation. While highly plausible, more information is required to substantiate this view.

Polymorphonuclear Leukocyte

As previously noted, several animal studies have demonstrated *in vivo* pulmonary recruitment of polymorphonuclear leukocytes (PMN) after airway challenge with various components of cotton (*26–28*). While similar effects follow the inhalation of pure endotoxin (*42*), the mechanisms are not necessarily the same. Skin biopsies from normal volunteers show an influx of cells including PMN 3 and 24 hr after intradermal injection of a cotton bract extract (*43*). In addition, various cotton preparations have *in vitro* PMN chemotactic properties (*29,44*). Ainsworth and Neuman (*29*) showed the major chemotaxin in cotton mill dust to be a water-soluble low molecular weight substance.

Once recruited, PMNs can release mediators affecting airway function. Such mediators include arachidonic acid products including leukotrienes. However, the amounts released are much less than those derived from mast cells, eosinophils, or AMs, and the major component is LTB_4, a chemotaxin for both PMN and eosinophils and also a weak bronchoconstrictor. Release of this leukotriene is probably important in amplifying the inflammatory response through further influx of PMN and eosinophils. PMNs also appear to release factors which increase mucus glycoprotein synthesis (*18*). Additionally, PMNs may play a role in a negative feedback mechanism in the metabolism of arachidonic end products. PMNs are a rich source of oxidizing free-radical products of oxygen, and these may inactivate bronchoconstrictors derived from arachidonic acid (*20*).

Thus, although PMNs are obviously important in the bronchitis associated with cotton or grain dust as they are prominently present histologically, their role in mediating airway reactivity is unclear. There have been no studies examining the effects of components of these dusts on PMN function except for the *in vitro* documentation that cotton dusts and their components are chemotactic for these cells. No studies of cotton or grain extracts on oxidant or protease production by these cells have been performed. Animal models of inducted bronchial reactivity in other systems, notably post-ozone exposure (*45*), have suggested an effector role of these cells in airway reactivity by showing diminished change in airway resistance to bronchial provocation after depletion of PMN. However, the effects of these methods of PMN depletion on other inflammatory mechanisms, such as cellular mediator release, are unclear. In summary, al-

though PMNs appear to influx into airways of subjects after exposure to cotton or grain dose, the function of these cells in mediating airway narrowing is unclear.

Mast Cells

These inflammatory cells are commonly implicated in the generation of bronchoconstricting mediators (histamine, leukotrienes, prostaglandins, serotonin, and kininogenase). These cells release an oligopeptide, termed prostaglandin-generating factor of anaphylaxis, which stimulates production of prostaglandins and HETEs from unidentified cells in exposed lung tissue (*46*). Mast cells also release several factors that increase mucus production (*18*). Release of histamine or a particular high molecular wieght neutrophil chemotactic factor (NCF-A) suggests mast cell degranulation has occurred (*11*), although basophils also release quantities of the former mediator. Arachidonic acid metabolites such as LTB_4 and HETEs are chemotactic lipids for PMNs and eosinophils released by mast cells; however, these are not unique to this cell, as already discussed. In addition, mast cells release unique factors chemotactic for eosinophils (eosinophil chemotactic factor of anaphylaxis, ECF-A) (*47*) and PMNs (neutrophil chemotactic factor of anaphylaxis, NCF-A) (*48*). The first group is a family of molecular weight 360 to 1000 preformed peptides, while the latter factor is a large protein, molecular weight (750,000), that has been associated with a variety of asthma syndromes. In addition, mast cells produce a number of proteolytic enzymes and reactive oxygen species that may damage airway epithelium or interact with other mediators (*49*). Mast cells have a granule-associated peroxidase enzyme, which may be important in inhibition of bronchoconstricting substances, as this particular enzyme has been shown to hydrolyze and inactivate a slow-reacting substance of anaphylaxis (SRS-A) *in vitro* (*50*). Finally, in contrast to the already described mast cell-derived chemotactic factors, mast cell-derived histamine has been shown to modulate negatively the activity of other immune cells, causing an inhibition of delayed hypersensitivity *in vitro* in a dose-dependent manner (*51*).

Although mast cell mediators are well studied, less is known about their importance in cotton- or grain-induced airway disease. Sodium cromoglycate, an inhibitor of mast cell degranulation, diminishes the bronchoconstriction occurring in mill workers exposed to cotton dust in the workplace (*52*). However, there is increasing evidence that this agent is not specific for mast cells. There are other indications that mast cells degranulate in association with exposure to cotton components. Histologic evidence of this has been seen after intradermal injection of a cotton bract extract in normal volunteers (*43*). In addition, chemical components of cotton dust including flavonols, flavonol glycosides and terpenoid aldehydes cause rat mast cell degranulation *in vitro* (*53,54*).

There is also biochemical evidence that mast cells are activated in cotton-associated airways disease. Several studies (*55,56*) have documented an increase in serum or urine histamine or histamine product levels in cotton workers. In addition, lung explant (*57,58*) and cell culture (*59*) experiments have shown release of histamine after exposure to aqueous cotton dusts. However, whether histamine is the only active bronchoconstricting agent in this disease is controversial as the kinetics of the airway response to cotton are different from those to histamine and antihistamines do not completely block the response (*2*). Instead, histamine may represent a marker for mast cell degranulation in association with exposure to cotton dust and may only be a part of the bronchoconstricting mechanisms.

In summary, because the mast cell has a unique pattern of mediator release, assessment of activation of this cell is less complicated than with other resident pulmonary cells. There is evidence that this cell is activated in association with exposure to cotton dust, as levels of one of these relatively specific mediators, histamine, has been shown to be increased in several studies. The role of other specific mast cell-derived mediators (e.g., neutrophil and eosinophil chemotactic factor of anaphylaxis) has not been studied to date in these dust related disorders.

Lymphocytes

These cells are primarily involved in humoral, cellular, and tumor immunology. Their relation to bronchoconstriction is unclear; only recent evidence has uncovered a potential bronchoconstricting function of these cells. T lymphocytes produce an antigen-binding factor that stimulates degranulation of mast cells much like IgE, resulting in liberation of numerous inflammatory mediators as already discussed (*60*). We have recently demonstrated (*61*) that the degree of reactivity to a cotton bract extract can be predicted by the percentage of lymphocytes in the bronchoalveolar lavage cellular differential performed several weeks prior to challenge with the bract extract. These findings suggest that resident pulmonary lymphocytes may be involved in modulation of the airway response to cotton constituents; how these cells modulate this response is unclear.

Eosinophils

The role of these cells in bronchospasm is not known, although they are commonly present in asthma. Factors chemotactic for eosinophils, such as leukotriene B4 and HETEs, are released by a variety of cells, including a unique specific eosinophil chemotactic factor from mast cells (*47*). There is some evidence that eosinophils serve an inhibitory role in bronchoconstriction as they secrete several factors that may inactivate mediators of bronchospasm. Eosinophils are rich in peroxidase which, as already noted, inactivates leukotrienes (SRS-A) when incubated with hydrogen peroxide and a halide (*50*). Eosinophils also contain other enzymes that may inactivate mast cell products. For example, arylsulfatase interferes with the biological activity of leukotrienes through ill-defined mechanisms (*62*). In addition, eosinophil phospholipases destroy platelet-activating factor and possibly

other bronchoconstricting lipid mediators (*63*). Finally, eosinophil histaminase inactivates histamine (*11*). A product of histamine inactivation, imidazolylacetic acid, activates eosinophils, enhancing complement receptor production and inducing chemotaxis of these cells (*11*). This may result in amplification of the effects of these cells.

All of these findings argue for an inhibitory role of eosinophils in bronchospasm. However, additional information suggests that these cells may enhance bronchial reactivity. Leukotriene concentrations produced by eosinophils on stimulation are significantly greater than those produced by stimulated PMNs, and the pattern consists primarily of bronchoconstricting leukotrienes such as LTC_4 (*20*), suggesting the eosinophils have bronchoconstricting properties of their own. In addition, products of peroxidase that have been shown to inactivate leukotrienes have also been shown paradoxically to stimulate mast cell degranulation with resultant production of more leukotrienes and other mediators (*64*). Thus, the effect of eosinophils on leukotriene production and activity is unclear. There have been no studies examining the role of eosinophils in cotton or grain associated airways disease. Peripheral or sputum eosinophilia has not been documented in grain or cotton mill workers. In addition, as noted, there have been no studies examining production of eosinophil-specific chemotactic factors in these diseases nor *in vitro* studies with these cells.

Platelets

One study (*65*) described acute thrombocytopenia in association with airway symptoms when workers were exposed to cotton dust, conceivably due to pulmonary platelet sequestration. Platelets secrete a number of bronchoconstricting mediators, including kinins and arachidonic acid metabolites (*11*). Release of these factors may be stimulated by the already mentioned platelet-activating factor or other platelet secretagogues. Components of cotton also induce release of these platelet-derived factors *in vitro* (*65,66*). Release of thromboxane, a bronchoconstricting prostaglandin, occurs in human platelet preparations exposed to cotton dust or bract extract (*66*). In addition, condensed tannins, chemical components of cotton, also induce release of thromboxane in these preparations and show synergistic effects with IgG in inducing platelet degranulation (*65*). In this latter study, the authors proposed that humoral reactions to bacteria in cotton and the endogenous platelet secretagogue activity of the cotton components result in stimulation of platelet release of this prostaglandin. This field of research may prove promising in the dissection of mechanisms of bronchoconstriction due to cotton dust. However, it is unclear how such *in vitro* findings can be interpreted into *in vivo* significance. For example, thrombotic thrombocytopenic purpura, a disease associated with platelet activation and pulmonary sequestration, does not result in signs of airway obstruction, suggesting *in vivo* platelet activation does not invariably result in bronchoconstriction.

Acellular Mechanisms

Complement

This cascade of serum proteins can be activated through two different pathways, either classical or alternative, both of which result in liberation of a number of protein products which modulate inflammatory cell activity. With complement activation the generation of anaphylatoxins, C3a and C5a, occurs. Both of these activation by-products have functions potentially important in mediating airway inflammation or bronchoconstriction (*67*). Both components bind to mast cells, thus stimulating release of histamine, and, in addition, C5a is chemotactic for PMN, potential cellular mediators of airway inflammation.

Components of both cotton and grain have been shown to activate complement both *in vitro* (*68–71*) and *in vivo* (*72*). Two studies (*68,69*) have shown crude preparations of grain to activate the alternative pathway of complement, while multiple studies (*70,71*) have shown both alternative and classical pathway activation by cotton dust extracts *in vitro*. In addition, Olenchock and colleagues (*72*) have shown evidence of *in vivo* activation of complement in normal human subjects after 6-hr exposure to cotton dust which did not correlate with endotoxin levels in the dust. Several studies (*1*) have failed to show *in vivo* signs of complement activation in subjects reacting to grain dust, either in the workplace or after bronchial challenge with grain preparations. Nonetheless, these studies suggest that both grain and cotton may exert some effects through activation of complement. As already discussed, the consequences of this may include release of histamine by mast cells and influx of PMN and eosinophils due to generation of chemotactic fragments. However, two dusts that do not cause bronchoconstriction, silica and asbestos, also activate complement *in vitro*, suggesting this may be a nonspecific effect and not necessarily related to bronchoconstriction.

Antibody Formation

In the lung, production of antigen-specific IgE antibodies may result in liberation of inflammatory mediators from macrophages or more importantly, mast cells, which result in bronchoconstriction or airway inflammation (*11*). IgG antibodies may also induce airway inflammation through activation of complement (*67*). There have been some studies suggesting a role for the humoral immune system in cotton-associated airway disease. Initial studies (*73*) suggested certain cotton workers developed precipitating antibodies, presumably IgG, to crude cotton dust extracts; however, subsequent studies proved this to be due to a nonspecific precipitating reaction of the cotton dust preparation (*74*). Serum precipitins to grain antigens have also been demonstrated in some symptomatic grain workers (*75*), but these do not correlate with severity of the disease. More recently, Jones and colleagues (*76*) have shown a correlation between serum IgE antibodies to crude extracts of cotton dust and decline in pulmonary function over a working day in workers

crushing cotton seed; confirmation awaits studies in cotton textile workers. The difficulty with any of these studies is the conglomerate of components in cotton dust, making identification of antibodies to specific antigens nearly impossible. Nonspecific effects of the cotton components also make testing for serum antibodies difficult. Arguing against the humoral system playing a crucial role in cotton dust-associated airway alterations is the finding in several studies that naive subjects may respond to an inhalational challenge with cotton extracts in a much shorter time period than is consistent with a humoral mechanism.

Summary

This review has attempted to summarize some groups of bronchoconstricting mechanisms and to indicate the present state of our knowledge of the relation between these mechanisms and the disorders due to these two vegetable dusts. It is apparent that in spite of the increasing number of inflammatory mediators, the general mechanisms of these disorders remain incompletely understood. Part of the difficulty derives from their complex interactions. Another problem lies in the lack of highly specific inhibitors, particularly of the lipoxygenase pathway, which might help dissect out specific mechanisms. Further, studies of asthma, and other airway disorders, in human subjects with the application of bronchoalveolar lavage (BAL) are just beginning and should help uncover mechanisms specific for humans exposed to these dusts.

Turning to the two vegetable dusts, these can cause predictable airway narrowing in selected individuals. The use of these dusts as a model for determining inflammatory mechanisms of airway alterations may prove to be enlightening. Analysis of the yearly cotton meetings supports this concept, as each year cellular and acellular mechanisms of cotton dust-derived airway alterations further predominate over epidemiologic studies. We feel the time has come for more human studies, either in normal subjects exposed to these respective dusts, or in cotton and grain workers. The site where most of the alterations associated with these dusts occurs, large and small airways, is readily accessible to BAL. We have found that during the acute airway response to an inhaled cotton bract extract BAL is entirely safe and easily performed. This procedure can yield airway cellular and fluid constituents that may be important in mediating bronchoconstricting effects. Perhaps, one day, the intricate processes which mediated airway tone will be uncovered through research in the field of cotton or grain dust airway alterations.

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